STUDIES ON THE INTEGUMENT OF THE SILKWORM, BOMBYX MORI. VII. THE PERMEABILITY OF THE INTEGUMENT TO OXYGEN AND CARBON DIOXIDE IN VIVO

TOSHIO ITO

Sericultural Experiment Station, Suginami-ku, Tokyo, Japan

The fact that arthropod cuticle is permeable to gases, such as O_2 or CO_2, has been known in various materials (von Buddenbrock and von Rohr, 1922; Maloeuf, 1936; Fraenkel and Herford, 1938; and see the review by Richards, 1951). But in the silkworm any research on this respect is not found. The experiments reported here were undertaken to get quantitative data on the permeability to O_2 and CO_2 in the silkworm and, further, to know whether or not there is some difference in this property between the larva and the pupa.

On the other hand, that the insect cuticle, especially the epicuticle, is waterproof is a well known fact in general (Wigglesworth, 1948; Richards, 1951) and a procedure, abrasion of the cuticle with alumina dust, has been employed in the study of the transpiration of water through the cuticle. In the present research the effect of the same treatment on the permeability to O_2 and CO_2 was examined. Further, some other effects of rubbing are described here preliminarily.

Materials and Methods

Two silkworm races, C122 × N122 and Taihei × Chôan, reared in the spring of 1952, were used as material. Oxygen uptake and CO_2 output were measured manometrically by Warburg apparatus with the materials operated as follows: (1) Normal larvae and pupae. The larvae were ligated immediately behind the head in order to avoid extrusion of the digestive fluid from the mouth, and at the level between the eighth and the ninth abdominal segment in order to prevent the excretion of excrements. (2) Sealed larvae and pupae. In the former all spiracles, and the parts of the body both anterior to the head ligation and posterior to the abdominal ligation were sealed with enamel paint, and in the latter all spiracles, the segments behind the seventh abdominal segment and the skin at the boundary between wings and body were sealed with it. Materials were placed in the vessels of the manometer 15 minutes after occlusion in the larva and 20 minutes after occlusion in the pupa. Readings were begun after a 15-minute equilibration period. Controls showed that the paint had lost its volatile components before measurements were made, and that neither the paint nor the ligation significantly affected the results. To measure CO_2 output, the method comparing O_2 uptake in the presence or absence of alkali was used. Besides 0.3 ml. of 10% KOH placed in the center well, 0.5 ml. of about 10% H_2SO_4 was placed in the side arm of the vessel for the purpose of taking up possible ammonia there. All experiments were carried out at 25° C. and the gas phase was air.

For certain experiments the surface of the cuticle was abraded by scattering alumina powder on hard paper and then rubbing the worm lightly against this repeatedly.

The time course of O_2 uptake did not show a linear relation but a slightly upward convex curve in the sealed worms, showing that the rate of O_2 uptake gradually decreased, as shown in Figure 1. This was also the case in CO_2 output. As it seemed possible to get more reliable and mutually comparable values in O_2 uptake and CO_2 output at the beginning of the measurement, μl. O_2 uptake or CO_2 output per gram live weight in the first 30 minutes was used as a measure of the amount of gas penetration through the skin. The number of sealed silkworms used varied from three to five for one vessel, and two to four measurements were repeated.

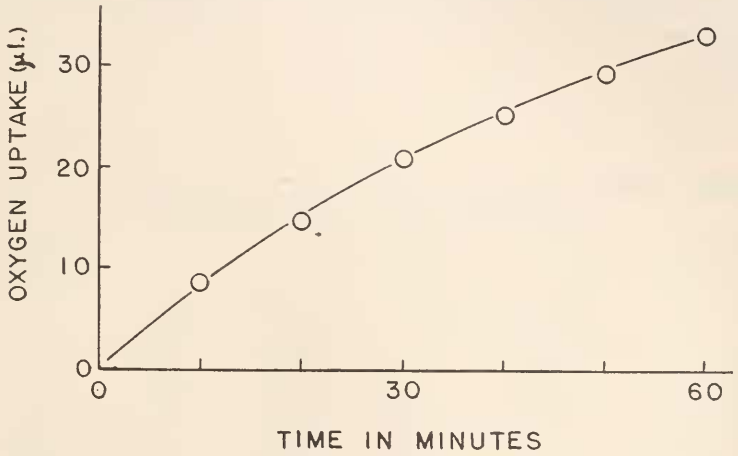

FIGURE 1. Time course of oxygen uptake of the closed silkworm larva. (μl./g. live weight).

It must be noted before the explanation of experimental results that there is no sure method of deciding whether the gasometric data obtained in the occlusion experiments are direct results of the gas penetration through the integument or not. But judging from the facts that the spiracles were perfectly occluded, that the film of the paint was considerably thick, and that there seems to be no part from which gases can penetrate out, it appears that the measured data in the occlusion experiments show practically the volume of gases penetrating through the integument.

OXYGEN UPTAKE AND CARBON DIOXIDE OUTPUT IN THE SEALED SILKWORM

The results of the experiments with fifth instar larvae as well as the pupae are given in Table I. From the values obtained in the sealed silkworms, it is obvious that the integument is permeable to O_2 and CO_2 both in the larva and pupa *in vivo*, even though the amount of gases is very much reduced in the sealed ones, to about one-fiftieth of the normals. Especially, after the middle time of the pupal stage, O_2 can be hardly measured. It has been already shown in the silkworm that the rate of the respiration varies during development (Itaya, 1940). This is shown also to be valid for the normal silkworms in Table I, where the respiratory rates similar to those obtained by him are represented, but not for the sealed ones. In other words, the rate of O_2 uptake or CO_2 output through the integument is per-

haps independent of the normal respiration. The values obtained in the sealed worms are relatively higher on the day of the larval and the pupal ecdysis, only, as compared with any other time. This may be caused by the fact that the cuticle is the thinnest at that time, both in the larva and pupa (Kuwana, 1933; Ito, 1951). However, such a phenomenon as the amount of gases passing through the integument being somewhat related to the thickness of the cuticle is not seen, after the first 24 hours of each ecdysis.

As shown in Table I, the values of the R. Q. are below 1.00 in the normal worms but considerably higher in the sealed ones (see also Table II), like those obtained in blowfly larvae by Fraenkel and Herford (1938). The R. Q. is distinctly different in the larva and the pupa, being 1.00 to 1.20 in the former and approximately 1.50 in the latter (one exception in the larva may be an experimental error).

TABLE I

Oxygen uptake, carbon dioxide output, and respiratory quotient in the normal and sealed silkworms (μl./30 minutes/g. live weight)

Stage	Normal silkworm			Sealed silkworm		
	O_2 uptake	CO_2 output	R. Q.	O_2 uptake	CO_2 output	R. Q.
1-day larva*	456	411	0.90	12.5	14.3	1.14
2-day larva	699	631	0.90	8.5	10.0	1.18
6-day larva	696	678	0.97	7.6	8.4	1.10
7-day larva	392	323	0.82	7.8	10.5	1.35
8-day larva†	428	360	0.84	9.2	9.2	1.00
10-day larva‡	241	205	0.85	9.4	10.0	1.06
1-day pupa§	190	168	0.88	12.2	19.1	1.57
4-day pupa	84	75	0.89	5.7	8.4	1.47
8-day pupa	179	165	0.92	±	11.2	—

* All larvae are in the 5th instar.
† The day of ripening (worms begin to spin cocoon).
‡ The third day after ripening.
§ Several hours after pupation.

Since the curve of the time course of O_2 uptake or CO_2 output in the sealed material is slightly convex, as shown above (Fig. 1), it is imagined that the rate of gas passage through the integument and the R. Q. value change according to the length of time after occlusion. The results of experiments in this connection are represented in Table II, in which the degree of change expressed by the ratio is also shown. In all cases the rate of O_2 uptake as well as of CO_2 output decreases and the ratio of decrease is more remarkable in the larva than in the pupa (see Fig. 2). The rate of O_2 uptake decreases less than that of CO_2 output and as a consequence the R. Q. becomes low both in the larva and pupa.[1] But several

[1] In the first-day pupa, however, somewhat increased R. Q. is gained. This seems to be caused by the presence of difficulty in manometer experiments in the time when the respiratory rate is very much depressed, in some time, to the extent of a scarcely recognizable value on the manometer.

hours after pupation (when the quinone-tanning process is not yet finished) the decrease is found relatively remarkable and is similar to that of the larva.

The rate of decrease shown in Table II is illustrated in Figure 2, in which all the data on the larval or pupal stage are collectively arranged according to the length of time after occlusion, as the degree of change in O_2 uptake or CO_2 output remains practically constant throughout larval or pupal stage. It is shown from this that the ratio decreases almost constantly according to the time after occlusion, regardless of stage. This tells us also that the amount of O_2 and CO_2 passing through the integument per gram live weight is nearly constant in the larva and pupa, respectively, except for the short period after the ecdysis, as mentioned above.

TABLE II

The change of oxygen uptake, carbon dioxide output, and respiratory quotient after occlusion of spiracles (μl./30 minutes/g. live weight)

Stage	Time in hours after occlusion*	Measured value			Ratio to the initial value (%)	
		O_2 uptake	CO_2 output	R. Q.	O_2 uptake	CO_2 output
2-day larva	0	8.5	10.0	1.18	100	100
	2	6.6	6.4	0.97	78	64
3-day larva	0	9.5	9.7	1.02	100	100
	3	4.2	3.6	0.86	44	37
5-day larva	0	11.8	12.3	1.04	100	100
	3	6.7	6.4	0.96	57	52
6-day larva	0	7.6	8.1	1.07	100	100
	1.5	6.4	5.9	0.92	84	73
1-day pupa	0	12.2	19.1	1.57	100	100
	2	8.4	14.8	1.76	59	77
4-day pupa	0	5.7	8.4	1.47	100	100
	2.5	5.2	6.7	1.29	91	80
8-day pupa	0	±	11.2	—	—	100
	3	—	9.0	—	—	80

* The second measurement of each stage was performed using the same worms.

MEASUREMENT IN THE RUBBED SILKWORM

The fact that the rate of transpiration of water is much increased when the most superficial layer of the insect cuticle is abraded by rubbing lightly with alumina dust is well established, but no information is available about the effect of rubbing on the permeability to gases. To know the effect of it in the silkworm, measurements were performed in the same way as in the preceding section, employing the worms rubbed with the powder immediately before spiracular sealing. The data are shown in Table III. From this it is obvious that when worms are rubbed, the rates of O_2 uptake and CO_2 output are increased as compared with

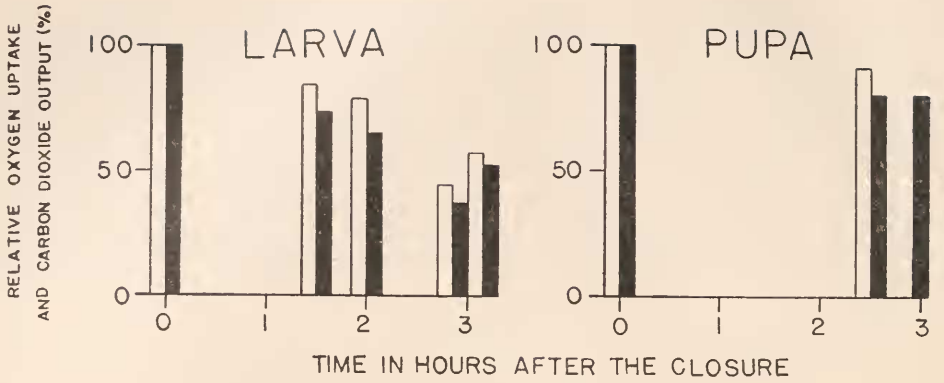

FIGURE 2. The decrease of oxygen uptake and carbon dioxide output of the sealed silkworm with the time lapse after closure (measurements for every 30 minutes). White column, oxygen uptake. Black column, carbon dioxide output.

the unrubbed worms (compare Table III with Table II). The increase of CO_2 output is proportionately one and a half times as high as the increase of O_2 uptake (Table IV). Further, the increase for both O_2 uptake and CO_2 output is a little higher in the larva than in the pupa, without exception. The fact that the increase of CO_2 output is higher than that of O_2 uptake throughout all experiments causes the value of the R. Q. to reach to a level as high as about 1.50 in the larva and more than 2.00 in the pupa.

The rate of O_2 uptake and CO_2 output decreases according to the time lapse after occlusion (Table III and Fig. 3), like the case mentioned above (Table II and Fig. 2). But the decrease of O_2 uptake is less marked than that of CO_2 output, especially in the larva. Therefore, reduction of the R.Q. value is remarkable in the larva.

TABLE III

Oxygen uptake, carbon dioxide output, and respiratory quotient of the silkworms rubbed on the cuticle with alumina dust (μl./30 minutes/g. live weight)

Stage	Time in hours after occlusion	Measured value			Ratio to the initial value (%)	
		O_2 uptake	CO_2 output	R. Q.	O_2 uptake	CO_2 output
3-day larva	0	25.6	36.1	1.42	100	100
	3	19.4	18.9	0.97	76	52
5-day larva	0	21.5	34.2	1.59	100	100
	3	19.0	16.9	0.89	88	49
1-day pupa	0	14.6	37.9	2.60	100	100
	2	10.8	23.9	2.21	74	63
4-day pupa	0	8.1	17.9	2.21	100	100
	2.5	7.2	13.3	1.85	89	74

TABLE IV

The ratio of increase of the rate of oxygen uptake as well as carbon dioxide output by abrasion in the sealed silkworms

Stage	O_2 uptake	CO_2 output
3-day larva	2.7	3.7
5-day larva	1.8	2.8
1-day pupa	1.2	2.0
4-day pupa	1.4	2.1

SOME REMARKS ON THE RUBBED SKIN

The cuticle begins to darken at the rubbed part a few hours after rubbing and becomes black or dark brown in color about 24 hours later. The color differs a little according to the different developmental stages and is similar to the color developed in the body fluid exposed to air. The darkening occurs both in the larva and pupa but there is some difference in the time before melanosis [2] according to the developmental stages. Another interesting fact observed in the rubbed integument is that the scales are not formed in the surviving adult, if the pupae are rubbed in the middle or later stage, though most of them die. However, if the rubbing is performed shortly before the larval or pupal ecdysis, the molted larva or pupa shows quite normal appearance. Histological observations will be described in the future.

DISCUSSION

From the occlusion experiment it is obvious that the integument of the larva or pupa in the silkworm is permeable to O_2 and CO_2 *in vivo*, at least in the sealed worms. There seems to be no direct way of deciding whether the cutaneous respiration reaches the observed value also in controls, but the opinion of Fraenkel and Herford (1938) that the amount of O_2 diffusing through the skin in the normal animals would be much less than in the closed animals, may be applied in the silk-

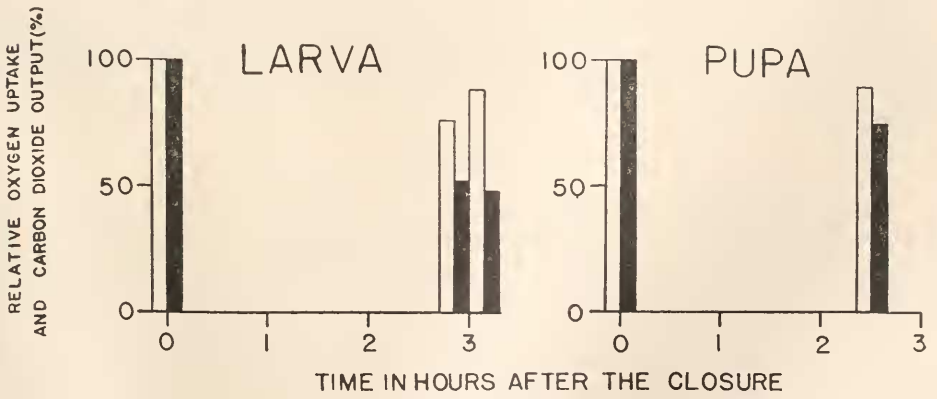

FIGURE 3. The decrease of oxygen uptake and carbon dioxide output of the rubbed and sealed silkworm with the time lapse after closure (measurements for every 30 minutes). The explanations are the same as for Figure 2.

[2] The darkening of the cuticle is considered to be a kind of melanosis.

worm also. And judging from the very low rate of O_2 uptake or CO_2 output in the sealed silkworms, that is, about one-fiftieth of the normals, it is sure that the cutaneous respiration is so slight as to be practically negligible as compared with the tracheal respiration.

In the larvae of *Calliphora erythrocephala* (Diptera), *Chearocampa elpenor* (Lepidoptera), *Tenebrio molitor* (Coleoptera) and *Culex* sp. (Diptera), the rates of respiration in the closed ones range from one-half to one-fifth of the normal animals (Fraenkel and Herford, 1938), being much higher than the values obtained in the silkworm (about one-fiftieth). This difference may be attributed to the minute structure of the cuticle, blood physiology and so forth. But O_2 uptake calculated from Table I amounts, for instance,

to 0.025 μl./mg./hr. (one-day larva of the fifth instar), or
to 0.015 μl./mg./hr. (six-day larva of the fifth instar)

in the closed worm in air, and these values do not differ, in the order, as compared with the values shown in *Calliphora* by the above two investigators. It is supposed, therefore, that the low value, such as one-fiftieth of the normal in the sealed silkworms, would be brought about because tracheal respiration is far more important in this insect than in *Calliphora erythrocephala,* etc.

In the closure experiment of *Cambarus bartoni* it is shown that the rate of respiration becomes lower with the time lapse, and that the R. Q. value becomes higher in this course (Maloeuf, 1936). In contrast to this, it is shown that the R. Q. value is reduced in the sealed silkworm, though the same change as in the former animal is seen also in the latter one as for the time course of the respiratory rate.

It was shown in other experiments that the transpiration of water is much increased by rubbing in the silkworm pupa (Ito and Tanaka, 1952). In this paper it is shown that the rate of permeability to O_2 and CO_2 is increased by rubbing, but the ratio of increase in permeability to O_2 and CO_2 by abrasion is far lower than that to water by the same procedure.

The author wishes to thank Prof. A. G. Richards of the University of Minnesota for reading the manuscript and his kind aid in connection with its publication. Thanks are also due to Drs. T. Yokoyama and Z. Kuwana for reading the manuscript.

Summary

1. From the results of measurements of O_2 uptake and CO_2 output in the sealed silkworm, it was confirmed that the gases penetrate through the integument both in the larva and pupa. The rate of O_2 uptake as well as CO_2 output is reduced to about one-fiftieth of the controls in the sealed silkworm. The R. Q. value of such silkworms ranged from 1.00 to 1.20 in the larva and was about 1.50 in the pupa. The rate of respiration became lower with the time lapse after occlusion, the decrease being more remarkable in the larva than in the pupa. Throughout the larval and pupal stages, the rate of O_2 uptake decreased less than that of CO_2 output.

2. The permeability of the skin rubbed just before occlusion increased a few times, the ratio being a little higher in the larva than in the pupa. The rate of in-

crease in CO_2 output was about one and one-half times that in O_2 uptake both in the larva and pupa. The decrease of the volume of measured gases seen with the time lapse in the rubbed material was not so remarkable in the pupa, and the rate of decrease in CO_2 output was relatively high in the larva. From this it was shown that the increase of cutaneous respiration by abrasion was not so remarkable as compared with that of water transpiration through the integument.

3. Some observations in relation to cuticle rubbing were briefly outlined.

LITERATURE CITED

von Buddenbrock, W., and G. von Rohr, 1922. Die Atmung von *Dixippus morosus*. *Zeitschr. allg. Physiol.*, **21**: 111–160.

Fraenkel, G., and G. V. B. Herford, 1938. The respiration of insects through the skin. *J. Exp. Biol.*, **15**: 266–280.

Itaya, K., 1940. Respiration and respiratory quotient in larva, pupa and adult of silkworm. *J. Sericul. Sci. Japan*, **11**: 113–140. (In Japanese.)

Ito, T., 1951. Studies on the integument of the silkworm, *Bombyx mori*. II. Histology and cytology of the integument at the pupation time. *Bull. Sericul. Exp. Sta.*, **13**: 585–611. (In Japanese with English summary).

Ito, T., and M. Tanaka, 1952. Waterproofing of the pupal cuticle in the silkworm. *Sanshi-kenkyû*, **2**: 23–26. (In Japanese.)

Kuwana, Z., 1933. Notes on the growth of cuticle in the silkworm. *Proc. Imp. Acad. Tokyo*, **9**: 280–283.

Maloeuf, N. S. R., 1936. Quantitative studies on the respiration of aquatic arthropods and on the permeability of their outer integument to gases. *J. Exp. Zool.*, **74**: 323–351.

Richards, A. G., 1951. The integument of arthropods. Univ. of Minnesota Press, Minneapolis.

Wigglesworth, V. B., 1948. The insect cuticle. *Biol. Rev.*, **23**: 408–451.